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Comparative Phytochemicals of Four *Heliotropium* Species to Determine Their Kinship Degree

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Abstract. In this study, four species of *Heliotropium* (Boraginaceae) were selected to determine their similarity using chemical compound content, as well as the effect of geographic location on this content. Three sample collection areas were selected, two in Basra Governorate and one in Dohuk Governorate. They were chemically studied using Gas Chromatography–Mass Spectrometry [GC-MS] to compare between them. 102 compound were isolated from *H. europaeum* , 61 compounds from *H. bacciferum* , 60 compounds from *H. crispum* and 167 compounds from *H. curassavicum*. Based on the similarity of phytochemicals, *H. bacciferum* and *H. crispum* were closely related, sharing 21 compounds. *H. europaeum* was the closest to them, sharing 12 compounds, whereas *H. curassavicum* was distantly related to *H. bacciferum* and *H. crispum* , sharing only three compounds with them. The study concluded that chemical analysis provides important taxonomic characteristics for species, as they can be separated based on their chemical content, and that the number of chemical compounds varies with the environment or geographic location.

Keywords. *Heliotropium*, GC-MS, Phytochemicals, Similarity, Boraginaceae family.

1. Introduction

There is a variety of phytochemical compounds produced by plants [1]. Researchers in plant chemistry have confirmed the possibility of using chemical content in plant taxonomy. Variation in this content among different species is one of the foundations used in plant taxonomy, as it provides support for other taxonomic characteristics [2].

Modern studies have become dependent on chemical studies to understand the phenotypic and evolutionary relationships between taxa, especially the study of chemical compounds formed as secondary metabolites, which can be identified with high efficiency using modern techniques. The structure and distribution of these compounds also have good taxonomic value for distinguishing between taxa, especially species of the same genus [3]. Several recent studies have used chemical content to compare genera or species, including the study of Grayer [4], Rønsted [5] and Rabemanolontsoa [6].

The genus *Heliotropium* belongs to the Boraginaceae family, one of the largest families of angiosperm dicotyledons in the world [7, 8]. It contains 248 species according to Kew [9], which are distributed worldwide because of their ability to tolerate a wide range of environmental conditions [10]. In Iraq, 18 species of this genus have been found [11] in the form of annual or perennial herbs or shrubs [12].

Species of this genus are used in traditional medicine in many countries for various diseases, and are still suitable enough for new medicines [13]. They are of medical importance because they contain alkaloids, flavonoids, phenols, naphthoquinones, terpenoids, phytosterols, glycosides, and tannins, which are also major secondary products of these plants [14].

These substances, found in many species, exhibit different biological and pharmacological activities, acting as antioxidants, antibacterial, antifungal, antiviral, antitumor, anti-inflammatory, and antimutagen properties [15, 16, 17]. They are also known to contain pyrrolizidine alkaloids, which are common secondary metabolites in the Boraginaceae family, including the genus Heliotropium.

The study aimed to determine the chemical content of four species of the genus *Heliotropium* [*H. europaeum, H. bacciferum, H. crispum* and *H. curassavicum*] found in locations with varying environmental conditions, using gas chromatography-mass spectrometry [GC-MS], and to identify chemical compounds shared by these species to determine the relationship between them.

2. Materials and Methods

2.1. Specimens Collection

The four studied plant species belonging to the genus *Heliotropium* were collected from the areas shown in Table 1 during their growing and flowering months [February - June 2024]. They were transferred to the laboratory at the Department of Life Sciences, College of Education for Pure Sciences, University of Basra, for morphological identification using an Olympus dendromic microscope. Species were identified based on Al-Rudainy [18], lowland plants of Iraq [19], plants of Turkey [20], and plants of Iran [21]. They were preserved in the herbarium of the College of Science, University of Basra, as reference samples. Some were also taken for chemical studies.

Table 1. Regions and periods of collection of the studied species.

Species	Collection region	Collection Periods
H. europaeum	The mountainous region of Dohuk	July
H. bacciferum	Basra Junction and Highway	November and December
H. crispum	Basra Junction and Highway	November and December
H. curassavicum	Siba and Mukhraq	October

2.2. Extraction of Chemical Compounds

Extraction was carried out for the four species according to Harborn [22]. The specimens were dried at laboratory temperature. The dried species were then ground separately into powder using electric grinder. Five grams of each powder were mixed with 250 ml of pure ethanol and the mixture was shaken well using a magnetic stirrer for 24 h at room temperature. It was then filtered using a filter paper. The filtrate was collected and placed in vacuum evaporator to evaporate the solvent. The extracts were then collected in Eppendorf tubes and sent to the Nehran Omar Laboratories of the Basra Oil Company for GC-MS analysis Type [Agilent /USA 7890 A].

3. Results

3.1. Mass Spectrum of Compounds Separated by Gas Chromatography-Mass Spectrometry [GC-MS]

The chemical study revealed clear differences in the distribution of chemical compounds among the studied species, both in the number and types of compounds.

3.2. Mass Spectrum of H. europaeum

A total of 102 chemical compounds were isolated by GC-MS from *H. europaeum*, shown in Table [2] and their different peaks are shown in Figure [1]. The active compound in this species was identified

as Butane, 1,1'-[oxybis[2,1-ethanediyloxy]]bis, because its area percentage was 13.325 % of the total area of the separated compounds .

Table 2. Chemical compounds that appeared in *H. eurpaeum*.

	RT	Name	Formula	Area	Area Pct
1	4.156	Propane, 2-fluoro-2-methyl-	C4H9F	10625232	0.5466943
2	4.435	2-Propenoic acid, ethenyl ester	C5H6O2	2731770	0.1405563
3	6.96	4,4-Dimethyl-thiazolidine	C5H11NS	2838098	0.1460271
4	7.025	Hydroxymethyl 2-hydroxy-2-methylpropionate	C6H12O4	18788262	0.9667023
5	7.174	1H-Imidazole-4-methanol	C4H6N2O	6357422	0.327105
6	7.208	3-Hexanol, 2-methyl-	C7H16O	4262236	0.2193025
7	7.736	2-Methyl-4-isopropyl-thiazolidine	C7H15NS	7271332	0.3741279
8	7.826	4-Cyclopentene-1,3-dione	C5H4O2	3543108	0.1823016
9	8.05	Diethylcyanamide	C5H10N2	6253203	0.3217427
10	8.244	1,4-Di[methylthio]-2-butene, [E]-	C6H12S2	6853755	0.3526425
11	8.404	2-Butenoic acid, 2-methyl-, [Z]-	C5H8O2	3047648	0.156809
12	9.033	Oxazole, 4,5-dihydro-2-methyl-	C4H7NO	3118933	0.1604768
13	9.543	Ethanedioic acid, dimethyl ester	C4H6O4	14088779	0.7249023
14	9.907	2,4-Dihydroxy-2,5-dimethyl-3[2H]-furan-3-one	C6H8O4	12333627	0.6345954
15	9.928	1H-Phosphole, 2,5-dihydro-1-methyl-	C5H9P	4037294	0.2077287
16	11.209	Phenylacetic acid, cyclobutyl ester	C12H14O2	7951829	0.4091411
17	11.407	l-Valine, N-methoxycarbonyl-, isohexyl ester	C13H25NO4	3299795	0.1697826
18	11.438	Indole	C8H7N	16721408	0.8603575
19	11.47	L-Valine, N-[methoxycarbonyl]-, methyl ester	C8H15NO4	18121772	0.9324097
20	11.777	Propanedioic acid, methyl-, dimethyl ester	C6H10O4	4319800	0.2222643
21	12.195	Aziridine, 2-phenyl-	C8H9N	17812997	0.9165225
22	12.222	Butanoic acid, 3,3-dimethyl-	C6H12O2	3164459	0.1628192
23	12.398	4-Octanol, 2,4-dimethyl-	C10H22O	7433642	0.3824792
24	12.986	1-Methyl-5-fluorouracil	C5H5FN2O2	3749397	0.1929157
25	13.205	Glycine, N-[ethoxycarbonyl]-, ethyl ester	C7H13NO4	4612090	0.2373034
26	14.025	Octanoic acid	C8H16O2	4403677	0.22658
27	14.081	Benzoic acid	C7H6O2	3243599	0.1668911
28	14.255	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	C6H6O4	48290819	2.4846813
29	14.394	2-[2-Oxo-2-[thiophen-2-yl]ethyl]propanedinitrile	C9H6N2OS	15178352	0.7809635
30	14.407	Butane, 1,1'-[oxybis[2,1-ethanediyloxy]]bis-	C12H26O3	258980610	13.3251889
31	14.5	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	C7H9NO2	4072088	0.2095189
32	14.566	Benzenamine, 2-chloro-4-methyl-	C7H8ClN	2748257	0.1414046
33	14.648	5-Hydroxymethylfurfural	C6H6O3	8252465	0.4246096
34	14.716	Cyclopentanone, 2-[1-methylpropyl]-	C9H16O	2941400	0.1513423
35	15.034	Benzeneacetic acid	C8H8O2	3550226	0.1826679
36	15.188	Glutaric acid, di[hexa-1,5-dien-3-yl] ester	C17H24O4	6003882	0.3089145
37	15.374	Butanoic acid, 2-ethyl-, 2-methylpropyl ester	C10H20O2	21252632	1.0935002
38	15.394	Oxalic acid mono-[N-methyl]-amide, ethyl ester	C5H9NO3	18629663	0.958542
39	15.407	N-Pentyloxazolidin-2,4-dione	C8H13NO3	247772748	12.7485169
40	15.414	2-Hydroxy-2,5-dimethyl-hept-6-en-3-one	C9H16O2	228112873	11.736968
41	15.478	1H-Pyrazole, 3-ethyl-4,5-dihydro-1,4-dimethyl-	C7H14N2	6515450	0.3352359
42	15.693	2-Propanol, 1-[isooctyloxy]-2-methyl-	C12H26O2	12386143	0.6372975
43	15.728	[R]-1-Ethyl-2-pyrrolidinecarboxamide	C7H14N2O	2824734	0.1453395
44	15.782	Phthalic acid, 2-phenylethyl propyl ester	C19H20O4	3455787	0.1778087
45	15.856	Carbonic acid, but-3-yn-1-yl tridecyl ester	C18H32O3	3588215	0.1846225
46	16.128	Eugenol	C10H12O2	10495495	0.540019
47	16.154	N-Methyl-3,5-dihydroxyaniline	C7H9NO2	2976630	0.1531549
48	16.209	n-Decanoic acid	C10H20O2	3064690	0.1576858
49	16.238	1H-Indene, 2,3,4,5,6,7-hexahydro-	C9H14	5555979	0.2858688
50	16.312	Pyrrolidine, 1-[2-methyl-1-butenyl]-	C9H17N	5601724	0.2882225

	RT	Name	Formula	Area	Area Pct
51	16.506	2-Cyclohexen-1-one, 3,5-dimethyl-	C8H12O	9784958	0.5034601
52	16.572	3-Methylbut-2-enoic acid, 4-nitrophenyl ester	C11H11NO4	8307543	0.4274435
53	16.614	2-Hydroxy-2,6-dimethyl-hept-6-en-3-one	C9H16O2	6867003	0.3533242
54	17.572	4H-Chromene, 4a,5,6,7,8,8a-hexahydro-2,3,5,5,8a-pentamethyl	C14H24O	4400593	0.2264213
55	17.581	Bicyclo[3.1.1]hept-2-ene, 6,6-dimethyl-2-[2-[phenylmethoxy]ethyl]-, [1S]-	C18H24O	4756426	0.2447298
56	18.053	Phenol, 3,5-bis[1,1-dimethylethyl]-	C14H22O	14186669	0.729939
57	18.217	[1R,5S]-rel-3-Ethyl-2,4-dioxa-3-phosphabicyclo[3.3.1]nonane 3-oxide	C8H15O3P	2353317	0.4881769
58	18.226	2-Hydroxy-1-[1'-pyrrolidiyl]-1-buten-3-one	C8H13NO2	14872357	0.7652193
59	18.488	1,4-Cyclohexadiene, 3,3,6,6-tetraethyl-	C14H24	4192727	0.2157261
60	18.5	2[4H]-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, [R]-	C11H16O2	7606801	0.3913886
61	18.625	Dodecanoic acid	C12H24O2	15705790	0.8081015
62	18.746	Benzene, 1,4-dimethoxy-	C8H10O2	6571103	0.3380994
63	18.762	Phenol, 4-ethenyl-2,6-dimethoxy-	C10H12O3	2757093	0.1418592
64	19.063	Pyrolo[3,2-d]pyrimidin-2,4[1H,3H]-dione	C6H5N3O2	4725900	0.2431592
65	19.624	2-Hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone	C9H12O2	2778333	0.1429521
66	20.031	.alpha.-Methyl-D-mannopyranoside	C7H14O6	3880851	0.1996793
67	20.198	4-Fluorobenzoic acid, tridec-2-ynyl ester	C20H27FO2	3461672	0.1781115
68	20.34	Benzamide, 4-fluoro-N-allyl-	C10H10FNO	681839	0.1853433
69	20.84	Tetradecanoic acid	C14H28O2	4328947	0.222735
70	21.199	Loliolide	C11H16O3	6858608	0.3528922
71	21.705	2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	2754503	0.1417259
72	21.864	Pentadecanoic acid	C15H30O2	4206196	0.2164191
73	22.037	1,2-Benzenedicarboxylic acid, bis[2-methylpropyl] ester	C16H22O4	12463035	0.6412538
74	22.055	Hexanedioic acid, mono[2-ethylhexyl]ester	C14H26O4	7230346	0.3720191
75	22.128	2-Butenoic acid, 2-methyl-, 2,3,5,7a-tetrahydro-7-[hydroxymethyl]-1H-pyrrolizin-1-yl ester, [1S-[1.alpha.[Z],7a.alpha.]]-	C13H19NO3	4705888	0.2421295
76	22.5	Hexadecanoic acid, methyl ester	C17H34O2	4357134	0.2241853
77	22.705	Carbonic acid, but-3-en-1-yl heptadecyl ester	C22H42O3	7417256	0.3816361
78	22.825	[5R,8aR]-5-Propyloctahydroindolizine	C11H21N	738447	0.2804203
79	22.976	Dibutyl phthalate	C16H22O4	9917454	0.5102774
80	22.991	n-Hexadecanoic acid	C16H32O2	112961935	5.8121692
81	23.159	Hexadecanoic acid, ethyl ester	C18H36O2	3769451	0.1939475
82	23.589	Borazine, 2,4,6-trimethyl-		99674023	5.1284735
83	23.94	.alpha.,.beta.-D-Glucopyranoside, 1-deoxy-1-dodecylthio-	C18H36O5S	13213864	0.6798858
84	24.065	3'-Acetyllycopsamine	C17H27NO6	3311956	0.1704083
85	24.18	13-Octadecenoic acid, methyl ester	C19H36O2	1688975	0.6413768
86	24.185	9-Octadecenoic acid [Z]-, methyl ester	C19H36O2	2691312	0.1384746
87	24.308	Phytol	C20H40O	3803401	0.1956943
88	24.592	6-Tridecanol, 3,9-diethyl-	C17H36O	20739736	1.0671104
89	24.635	9-Octadecenoic acid [Z]-, octyl ester	C26H50O2	14997680	0.7716675
90	24.745	Linoleic acid ethyl ester	C20H36O2	7267977	0.3739553
91	24.792	9-Octadecenoic acid, ethyl ester	C20H38O2	7634649	0.3928215
92	24.795	Octadecanoic acid	C18H36O2	5145418	0.2647444
93	25.05	Butanoic acid, 2-hydroxy-2-[1-methoxyethyl]-3-methyl-, [2,3,5,7a-tetrahydro-1-hydroxy-1H-pyrrolizin-7-yl]methyl ester, [1S-[1.alpha.,7[2R*,3S*],7a.alpha.]]-	C16H27NO5	71051067	3.655752
94	26.208	N-Cyclopentyl-1-[2-methoxyethyl]-2-methyl-5-oxopyrrolidine-2-carboxamide	C14H24N2O3	3010867	0.1549165

	RT	Name	Formula	Area	Area Pct
95	26.573	Phenol, 3-[dimethylamino]-	C8H11NO	2641762	0.1359251
96	26.742	Hexanedioic acid, bis[2-ethylhexyl] ester	C22H42O4	3642492	0.1874152
97	28.094	4-Iodo-1H-pyrazole-3-carbonitrile, N-methyl	C5H4IN3	9153977	0.4709946
98	28.384	2-Anilino-4-methylquinoline	C16H14N2	35245638	1.8134747
99	28.624	Echimidine	C20H31NO7	74153840	3.8153973
100	29.035	9,12-Octadecadienoic acid [Z,Z]-, 2-hydroxy-1-[hydroxymethyl]ethyl ester	C21H38O4	11012706	0.5666308
101	29.1	Methyl 9,10-octadecadienoate	C19H34O2	4049366	0.2083498
102	34.585	.gamma.-Sitosterol	C29H50O	10802485	0.5558144

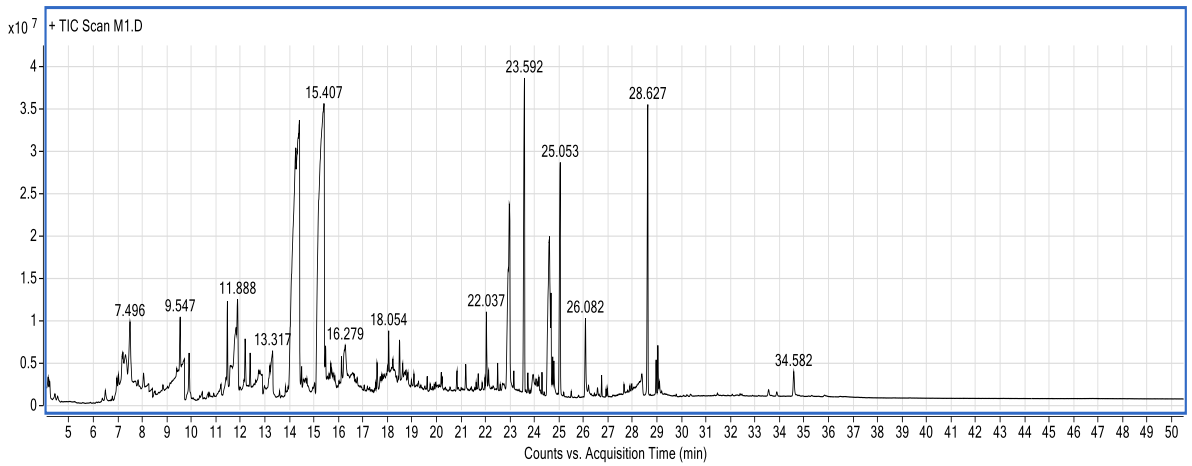

Figure 1. Mass spectrum of separated compounds of *H. europaeum* by GC-MS

3.3. Mass Spectrum of H. bacciferum

A total of 61 chemical compounds were isolated by GC-MS from *H. bacciferum*, shown in Table 3 and their different peaks are shown in Figure 2. The active compound in this species was identified as 2,3-Butanediol, 2,3-dimethyl--, because its area percentage reached 27.25 % of the total area of the separated compounds.

Table 3. Chemical compounds that appeared in *H. bacciferum* .

	RT	Name	Formula	Area	Area Pct
1	6.724	Butanoic acid, 2-oxo-, methyl ester	C5H8O3	1967474	0.7471349
2	7.577	Hydroxymethyl 2-hydroxy-2-methylpropionate	C6H12O4	3561743	1.3525478
3	8.043	L-Valinamide, N-[methoxycarbonyl]-	C7H14N2O3	2015841	0.765502
4	9.398	[S]-[+]-5-Methyl-1-heptanol	C8H18O	751897	0.2855278
5	10.144	Heptane, 2,2,3,3,5,6,6-heptamethyl-	C14H30	955394	0.3628044
6	10.51	Propane, 2-fluoro-2-methyl-	C4H9F	1488682	0.5653169
7	10.775	D-Limonene	C10H16	1268753	0.4818004
8	11.763	Indole	C8H7N	5329542	2.0238575
9	12.182	Aziridine, 2-phenyl-	C8H9N	3150652	1.1964388
10	12.937	1-Methyl-5-fluorouracil	C5H5FN2O2	2423260	0.502686
11	14.076	2,3-Butanediol, 2,3-dimethyl-	C6H14O2	71760606	27.2506048
12	14.08	Benzoic acid	C7H6O2	3243599	0.1668911
13	14.098	Benzofuran, 2,3-dihydro-	C8H8O	822127	0.3121972
14	14.12	Silane, dichloromethylpentyl-	C6H14Cl2Si	1643376	0.3409053
15	15.019	2-Hydroxy-2,6-dimethyl-hept-6-en-3-one	C9H16O2	25836675	9.8113026
16	15.357	Ethanamine, 2-methoxy-N-[2-methoxyethyl]-N-methyl-	C7H17NO2	1040650	0.3951798
17	15.656	2-[Hydroxymethyl]-6-methylpyridin-3-ol	C7H9NO2	4453539	1.6912013
18	15.728	Phthalic acid, ethyl 3-phenylpropyl ester	C19H20O4	3862688	1.4668296
19	15.771	Phthalic acid, butyl 3-phenylpropyl ester	C21H24O4	3647394	1.3850732
20	15.79	Benzoic acid, 4-amino-, methyl ester	C8H9NO2	2725871	1.0351311
21	15.864	Piperidin-2-one-5-carboxylic acid, 5,6-	C7H9NO3	1003268	0.3809842

	RT	Name	Formula	Area	Area Pct
		didehydro-, methyl[ester]			
22	16.101	n-Decanoic acid	C10H20O2	1120077	0.4253417
23	16.308	1-Methyl-3-acetylindole	C11H11NO	1997320	0.7584688
24	16.351	L-Glutamic acid, N,N-dimethyl-, diethyl ester	C11H21NO4	2223703	0.8444362
25	16.995	Benzeneethanol, 2-amino-6-methoxy-	C9H13NO2	2692896	1.022609
26	17.222	N,N'-Bis[2,6-dimethyl-6-nitrosohept-2-en-4-one]	C18H30N2O4	3223244	1.2240051
27	16.277	6-Azathymine, Bis[methyl] ether	C6H9N3O2	20488278	1.0541723
28	17.313	3-Ethyl-2,6,10-trimethylundecane	C16H34	787143	0.2989122
29	17.341	1H-Pyrrolizine, 2,3-dihydro-1-methoxy-7-[methoxymethyl]-	C10H15NO2	2879974	1.0936506
30	17.607	2-Ethoxy-4-methylaniline	C9H13NO	2011778	0.7639591
31	18.03	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	C19H30O3	1516306	0.5758069
32	18.179	Heptyl methyl methylphosphonate	C9H21O3P	4089431	1.5529338
33	18.19	1H-Pyrrolizine-7-methanol, 2,3,5,7a-tetrahydro-1-hydroxy-, [1S-cis]-	C8H13NO2	6157615	2.3383126
34	18.453	2[4H]-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, [R]-	C11H16O2	1005186	0.3817126
35	18.578	Dodecanoic acid	C12H24O2	1575643	0.3268546
36	19.178	Octahydropyrrolo[1,2-a]pyrazine	C7H14N2	1438765	0.5463613
37	20.345	Benzamide, 4-fluoro-N-allyl-	C10H10FNO	681839	0.1853433
38	20.81	Tetradecanoic acid	C14H28O2	1444719	0.5486223
39	21.141	Loliolide	C11H16O3	1465382	0.5564689
40	21.699	2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	1813722	0.6887487
41	22.025	Phthalic acid, hept-4-yl isobutyl ester	C19H28O4	2401395	0.9119135
42	22.031	1,2-Benzenedicarboxylic acid, bis[2-methylpropyl] ester	C16H22O4	4519946	1.2286506
43	22.496	Hexadecanoic acid, methyl ester	C17H34O2	2037723	0.7738115
44	22.891	n-Hexadecanoic acid	C16H32O2	18089293	6.8692867
45	22.965	Dibutyl phthalate	C16H22O4	1562286	0.5932675
46	23.501	Borazine, 2,4,6-trimethyl-	C3H12B3N3	21436244	8.1402687
47	23.667	3-Pyridinamine, 2,6-dimethyl-	C7H10N2	3835531	1.456517
48	23.923	3'-Acetyllycopsamine	C17H27NO6	1998677	0.7589841
49	24.18	13-Octadecenoic acid, methyl ester	C19H36O2	1688975	0.6413768
50	24.306	Phytol	C20H40O	1806625	0.6860536
51	24.141	4,6-Diamino-O-cresol	C7H10N2O	3688728	0.1897941
52	24.502	Bicyclo[3.2.1]octane	C8H14	1333305	0.5063136
53	24.539	9-Octadecenoic acid, [E]-	C18H34O2	3671862	1.3943648
54	24.572	Tricyclo[4.3.1.0[2,5]]decane	C10H16	886356	0.3365877
55	24.588	9,12,15-Octadecatrienoic acid, [Z,Z,Z]-	C18H30O2	3898618	0.8087372
56	24.61	cis-7,cis-11-Hexadecadien-1-yl acetate	C18H32O2	78778208	4.0533324
57	24.721	Octadecanoic acid	C18H36O2	1117982	0.4245461
58	24.939	Butanoic acid, 2-hydroxy-2-[1-methoxyethyl]-3-methyl-, [2,3,5,7a-tetrahydro-1-hydroxy-1H-pyrrolizin-7-yl]methyl ester, [1S-[1.alpha.,7[2R*,3S*],7a.alpha.]]-	C16H27NO5	23114814	8.7776943
59	26.737	Hexanedioic acid, bis[2-ethylhexyl] ester	C22H42O4	762373	0.289506
60	31.488	4-Methyl-2,4-bis[p-hydroxyphenyl]pent-1-ene, 2TMS derivative	C24H36O2Si2	963107	0.3657334
61	34.573	.gamma.-Sitosterol	C29H50O	4380777	1.6635704

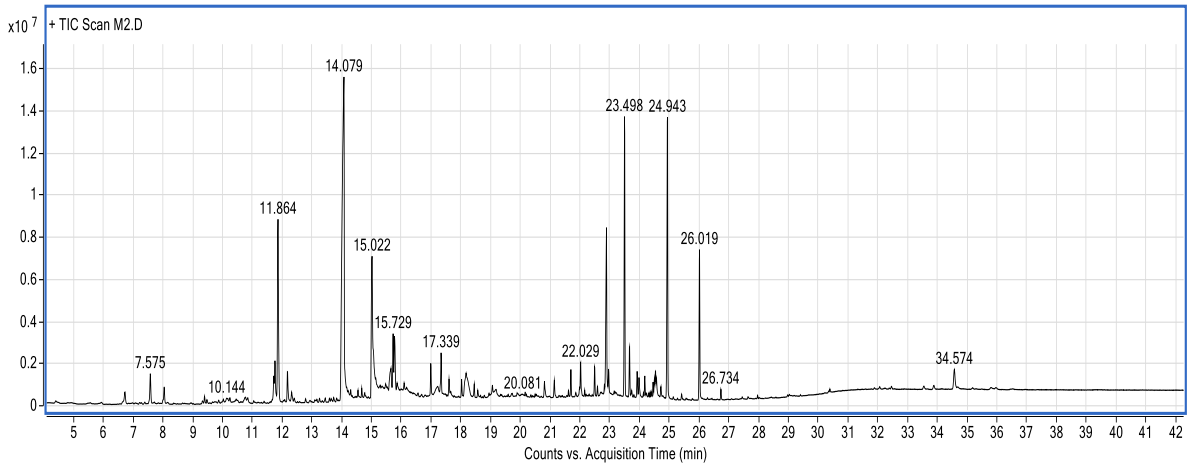

Figure 2. Mass spectrum of separated compounds of *H. bacciferum* by GC-MS.

3.4. Mass Spectrum of H. crispum

A total of 60 chemical compounds were isolated by GC-MS from *H. crispum*, shown in Table 4 and their different peaks are shown in Figure 3. The active compound in this species was identified as 2,3-Butanediol, 2,3-dimethyl--, because its area percentage reached 29.031 % of the total area of the separated compounds.

Table 4. Chemical compounds that appeared in *H. crispum*.

	RT	Name	Formula	Area	Area Pct
1	4.393	1,3,5,7-Tetroxane	C4H8O4	2130066	0.4418652
2	6.724	Butanoic acid, 2-oxo-, methyl ester	C5H8O3	1967474	0.7471349
3	7.551	Hydroxymethyl 2-hydroxy-2-methylpropionate	C6H12O4	1579531	0.3276611
4	8.04	L-Valinamide, N-[methoxycarbonyl]-	C7H14N2O3	2015841	0.765502
5	10.762	Bicyclo[3.2.1]octane	C8H14	656045	0.1783318
6	10.856	D-Limonene	C10H16	2550724	0.5291274
7	11.423	Propane, 2-fluoro-2-methyl-	C4H9F	10540740	2.1865926
8	11.612	Indole	C8H7N	8127441	1.6859729
9	12.196	Aziridine, 2-phenyl-	C8H9N	1420018	0.2945714
10	12.937	1-Methyl-5-fluorouracil	C5H5FN2O2	2423260	0.502686
11	14.07	Benzoic acid	C7H6O2	3243599	0.1668911
12	14.12	Silane, dichloromethylpentyl-	C6H14Cl2Si	1643376	0.3409053
13	14.138	2,3-Butanediol, 2,3-dimethyl-	C6H14O2	139947305	29.0309537
14	15.059	2-Hydroxy-2,6-dimethyl-hept-6-en-3-one	C9H16O2	41752991	8.6613254
15	15.194	2-Hexanol, 2-methyl-	C7H16O	11316064	2.3474273
16	15.398	Hydrazine, 1-methyl-1-[2-methylpropyl]-	C5H14N2	2617644	0.5430094
17	15.673	2-[Hydroxymethyl]-6-methylpyridin-3-ol	C7H9NO2	4228373	0.8771423
18	15.734	Phthalic acid, butyl 3-phenylpropyl ester	C21H24O4	5290399	1.0974511
19	15.777	Phthalic acid, hexyl 3-phenylpropyl ester	C23H28O4	2321907	0.4816611
20	15.798	Benzoic acid, 4-amino-, methyl ester	C8H9NO2	3203168	0.6644717
21	15.927	6-Azathymine, Bis[methyl] ether	C6H9N3O2	2389432	0.4956686
22	16.10	n-Decanoic acid	C10H20O2	1120077	0.4253417
23	17.002	Benzeneethanol, 2-amino-6-methoxy-	C9H13NO2	3469154	0.9430153

	RT	Name	Formula	Area	Area Pct
24	17.195	4,4-Dimethyl-2-pentanol, chlorodifluoroacetate	C9H15ClF2O2	4871937	1.0106445
25	17.221	N,N'-Bis[2,6-dimethyl-6-nitrosohept-2-en-4-one]	C18H30N2O4	3223244	1.2240051
26	17.314	3-Ethyl-2,6,10-trimethylundecane	C16H34	3369047	0.9158034
27	17.346	1H-Pyrrolizine, 2,3-dihydro-1-methoxy-7-[methoxymethyl]-	C10H15NO2	4139624	0.858732
28	17.622	2-Ethoxy-4-methylaniline	C9H13NO	678734	0.1844993
29	18.035	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	C19H30O3	1595226	0.3309169
30	18.15	1H-Pyrrolizine-7-methanol, 2,3,5,7a-tetrahydro-1-hydroxy-, [1S-cis]-	C8H13NO2	6157615	2.3383126
31	18.461	2[4H]-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, [R]-	C11H16O2	3835928	1.042715
32	18.582	Dodecanoic acid	C12H24O2	1575643	0.3268546
33	20.345	Benzamide, 4-fluoro-N-allyl-	C10H10FNO	681839	0.1853433
34	20.816	Tetradecanoic acid	C14H28O2	2371987	0.4920498
35	21.149	Loliolide	C11H16O3	3166275	0.6568185
36	21.708	2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	15876209	4.3156078
37	22.029	1,2-Benzenedicarboxylic acid, bis[2-methylpropyl] ester	C16H22O4	1447012	0.3001711
38	22.5	Hexadecanoic acid, methyl ester	C17H34O2	3117344	0.6466682
39	22.89	4-Methoxyphenyl pentyl ether	C12H18O2	6385308	1.3245813
40	22.915	n-Hexadecanoic acid	C16H32O2	35450072	7.3538351
41	22.97	Dibutyl phthalate	C16H22O4	1705868	0.3538687
42	23.667	3-Pyridinamine, 2,6-dimethyl-	C7H10N2	3835531	1.456517
43	23.589	Borazine, 2,4,6-trimethyl-	C3H12B3N3	99674023	20.6765821
44	23.667	3-Pyridinamine, 2,6-dimethyl-	C7H10N2	3835531	1.456517
45	23.903	3'-Acetyllycopsamine	C17H27NO6	1697657	0.3521654
46	24.185	13-Octadecenoic acid, methyl ester	C19H36O2	2975822	0.6173106
47	24.306	Phytol	C20H40O	1838685	0.3814206
48	24.524	cis-7,cis-11-Hexadecadien-1-yl acetate	C18H32O2	7715210	1.6004589
49	24.531	9-Octadecenoic acid, [E]-	C18H34O2	3671862	1.3943648
50	24.56	1-Heptanol, 2,4-diethyl-	C11H24O	6300782	1.307047
51	24.572	Tricyclo[4.3.1.0[2,5]]decane	C10H16	886356	0.3365877
52	24.593	9,12,15-Octadecatrienoic acid, [Z,Z,Z]-	C18H30O2	3898618	0.8087372
53	24.729	Octadecanoic acid	C18H36O2	1861440	0.3861409
54	24.945	Butanoic acid, 2-hydroxy-2-[1-methoxyethyl]-3-methyl-, [2,3,5,7a-tetrahydro-1-hydroxy-1H-pyrrolizin-7-yl]methyl ester, [1S-[1.alpha.,7[2R*,3S*],7a.alpha.]]-	C16H27NO5	20512100	4.2550718
55	26.015	4,6-Diamino-O-cresol	C7H10N2O	4139808	0.8587702
56	26.737	Hexanedioic acid, bis[2-ethylhexyl] ester	C22H42O4	762373	0.289506
57	31.54	4-Methyl-2,4-bis[p-hydroxyphenyl]pent-1-ene, 2TMS derivative	C24H36O2Si2	6815856	1.413895
58	34.587	.gamma.-Sitosterol	C29H50O	8052217	1.6703683
59	38.774	4-Fluorobenzylamine, N,N-dinonyl-	C25H44FN	1822187	0.3779982
60	38.812	Fumaric acid, di[2,2,3,3,4,4,5,5-octafluoropentyl] ester	C14H8F16O4	3095919	0.6422237

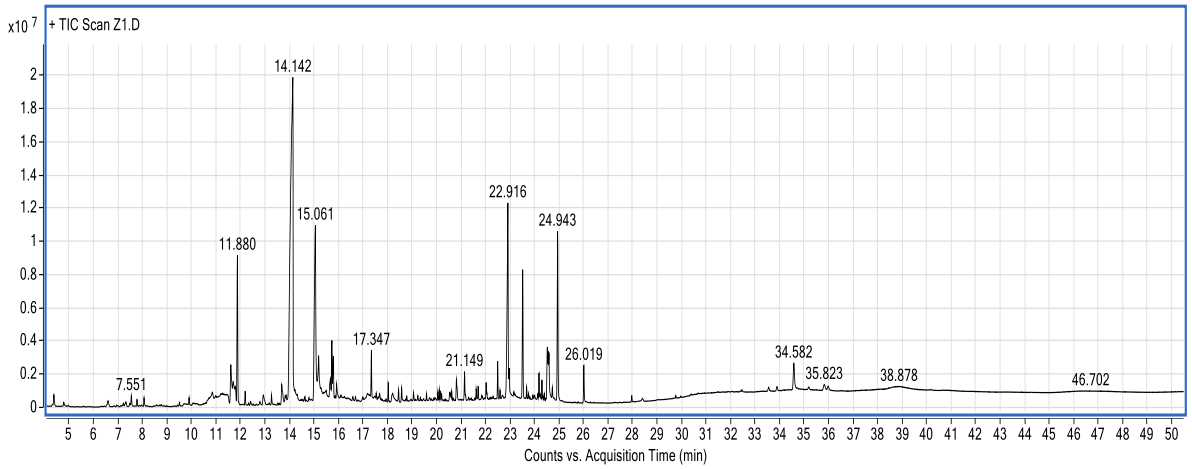

Figure 3. Mass spectrum of separated compounds of *H. crispum* by GC-MS.

3.5. Mass Spectrum of H. curassavicum

A total of 167 chemical compounds were isolated by GC-MS from *H. curassavicum*, shown in Table 5 and their different peaks are shown in Figure [4]. The active compound in this species was identified as 4[1H]-Pyrimidinone, 2,6-dimethyl-, because its area percentage reached 11.892 % of the total area of the separated compounds.

Table 5. Chemical compounds that appeared in *H. curassavicum*.

	RT	Name	Formula	Area	Area Pct
1	4.896	L-Valinamide, N[2],N[2]-dimethyl-	C7H16N2O	1677786	0.4560702
2	5.2	2-Propanone, ethylhydrazone	C5H12N2	2122549	0.5769695
3	5.545	6-Hepten-3-one, 5-hydroxy-4-methyl-	C8H14O2	926140	0.2517513
4	6.501	Ethene, 1,1-dichloro-	C2H2Cl2	639526	0.1738415
5	6.806	2-[1,3-Thiazol-4-yl]ethanamine	C5H8N2S	504734	0.1372011
6	7.213	3-Hexanol, 2-methyl-	C7H16O	671114	0.182428
7	8.296	N,N-Dimethyl-4-piperidinamine	C7H16N2	1595302	0.4336487
8	9.413	Cyclopentane, 1,1,3-trimethyl-	C8H16	1304579	0.3546219
9	9.668	N-[3-Phenylbutyl]-undecafluior-hexanamide	C16H14F11NO	499822	0.1358659
10	9.718	tert-Butyldimethylsilanol	C6H16OSi	1084851	0.2948935
11	9.9	2,4-Dihydroxy-2,5-dimethyl-3[2H]-furan-3-one	C6H8O4	592555	0.1610734
12	9.982	Succinic acid, monochloride 2-methylpent-3-yl ester	C10H17ClO3	580689	0.1578479
13	10.133	.beta.-l-Arabinopyranoside, methyl	C6H12O5	2993335	0.8136741
14	10.255	5-Hydroxypentanal	C5H10O2	440609	0.1197701
15	10.349	2-Ethylbutyl [E]-2-methylbut-2-enoate	C11H20O2	642526	0.1746569
16	10.478	But-3-enyl [E]-2-methylbut-2-enoate	C9H14O2	511763	0.1391118
17	10.622	5-Chloropentanoic acid, 2-tetrahydrofurylmethyl ester	C10H17ClO3	2512288	0.6829118
18	10.641	Ethanone, 1-[2-methyl-2-cyclopenten-1-yl]-	C8H12O	2549784	0.6931042
19	10.814	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-[1-methylethyl]-	C10H16	2037882	0.5539546
20	10.884	1H-Pyrazole, 4,5-dihydro-3,4,5-trimethyl-	C6H12N2	546342	0.1485114
21	11.062	3-Octen-2-one	C8H14O	1565260	0.4254824
22	11.199	4-Methyl-6-hepten-4-olide	C8H12O2	552260	0.1501201
23	11.405	2-Octenal, [E]-	C8H14O	968286	0.2632078
24	11.489	Indole	C8H7N	1972701	0.5362366
25	11.628	Benzene, 1-isocyano-4-methyl-	C8H7N	3973623	1.0801444
26	11.944	[1-Isopropylcyclobutyl]methylamine	C8H17N	773028	0.2101311
27	12.066	2-Methylhept-6-en-3-one	C8H14O	561719	0.1526913
28	12.112	cis-1-Ethoxy-1-butene	C6H12O	1325633	0.360345

	RT	Name	Formula	Area	Area Pct
29	12.194	Aziridine, 2-phenyl-	C8H9N	941022	0.2557967
30	12.2	Nonanal	C9H18O	730784	0.198648
31	12.248	6-Methyl-3,5-heptadiene-2-one	C8H12O	766743	0.2084227
32	12.267	cis-3-Hexenyl isovalerate	C11H20O2	532127	0.1446473
33	12.302	5-Ethyl-2-hepten-4-one	C9H16O	997273	0.2710873
34	12.36	1H-Imidazole, 4-methyl-	C4H6N2	572651	0.1556629
35	12.427	2,2'-Bi-2H-pyran, octahydro-	C10H18O2	732783	0.1991914
36	12.531	2-Heptenoic acid	C7H12O2	1801102	0.4895911
37	12.743	Succinimide	C4H5NO2	1201786	0.3266798
38	12.839	3-Cyclohexyloxypropylamine, N,N-dimethyl-	C11H23NO	721431	0.1961056
39	12.954	1-Methyl-5-fluorouracil	C5H5FN2O2	1391398	0.3782218
40	12.99	1-Adamantanol	C10H16O	510149	0.1386731
41	13.116	Thiazole, 5-methyl-	C4H5NS	737577	0.2004945
42	13.356	9-Ethylbicyclo[3.3.1]nonan-9-ol	C11H20O	832105	0.2261899
43	13.424	Heptanediamide, N,N'-di-benzoyloxy-	C21H22N2O6	1503441	0.4086783
44	13.595	Ethanone, 1-[4-methylphenyl]-	C9H10O	4949124	1.3453135
45	13.657	Benzenethiol, 3-methyl-	C7H8S	882320	0.2398398
46	13.683	Butanoic acid, 2-methylcyclohexyl ester, cis-	C11H20O2	1173130	0.3188903
47	13.758	trans-2,4-Dimethylthiane, S,S-dioxide	C7H14O2S	1626352	0.442089
48	13.828	1-Nonylcycloheptane	C16H32	635759	0.1728175
49	14.018	Cyclohexylmethylsilane	C7H16Si	1503053	0.4085728
50	14.118	Isopulegol	C10H18O	3268425	0.8884514
51	14.21	Benzenemethanol, .alpha.,4-dimethyl-	C9H12O	582261	0.1582752
52	14.309	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	C7H9NO2	3319627	0.9023696
53	14.472	Cyclobutanecarboxylic acid, 3-methylbutyl ester	C10H18O2	549548	0.1493829
54	14.577	Phenol, 4-methoxy-3-methyl-	C8H10O2	2361850	0.6420184
55	14.614	Benzeneacetic acid	C8H8O2	1154956	0.3139501
56	14.679	2-Decenal, [E]-	C10H18O	5233513	1.4226186
57	14.766	cis-1-Hydroxybicyclo[4.4.0]decane	C10H18O	492961	0.1340009
58	14.81	Nonanoic acid	C9H18O2	2444398	0.6644573
59	14.895	3-trsns-[1,1-dimethylethyl]-4-trans-methoxycyclohexanol	C11H22O2	663715	0.1804167
60	15.013	3-Ethyl-5-methyl-1-heptyn-3-ol	C10H18O	487669	0.1325624
61	15.069	Tricyclo[3.2.1.0[2,4]]octan-8-one, 3,3-dimethyl-, [1.alpha.,2.alpha.,4.alpha.,5.alpha.]-	C10H14O	475949	0.1293766
62	15.148	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, [1S]-	C10H16O	1895123	0.5151486
63	15.476	2,4-Decadienal, [E,E]-	C10H16O	930387	0.2529058
64	15.516	Divinylmethyl[N-methylcarbamoyloxymethyl]silane	C8H15NO2Si	623112	0.1693797
65	15.538	2-Cyclopenten-1-one, 2,3,4-trimethyl-	C8H12O	511868	0.1391404
66	15.709	1H-Indole, 1,3-dimethyl-	C10H11N	582773	0.1584144
67	15.728	Phthalic acid, butyl 3-phenylpropyl ester	C21H24O4	4378228	1.1901276
68	15.79	Benzaldehyde, 3-methoxy-, oxime	C8H9NO2	1176998	0.3199417
69	15.881	2[1H]-Pyridinone, 4-hydroxy-6-methyl-	C6H7NO2	1065019	0.2895026
70	15.954	Nonane, 2,2,4,4,6,8,8-heptamethyl-	C16H34	482029	0.1310293
71	15.982	1H-Pyrrole-3-carboxylic acid, N-methyl-, methyl ester	C7H9NO2	483132	0.1313291
72	16.012	L-Menthone	C10H18O	1109677	0.301642
73	16.105	2-Undecenal	C11H20O	2057005	0.5591528
74	16.175	5-Ethylcyclopent-1-enecarboxaldehyde	C8H12O	941214	0.2558489
75	16.315	1-Benzoxepin-2[3H]-one, octahydro-	C10H16O2	494370	0.1343839
76	16.383	N-Ethyl-2-isopropoxycarbonylazetidine	C9H17NO2	583323	0.1585639
77	16.434	3-Methyl-2-butenoic acid, but-3-yn-2-yl ester	C9H12O2	516901	0.1405085
78	16.531	Flamenol	C7H8O3	586325	0.1593799

	RT	Name	Formula	Area	Area Pct
79	16.58	[S]-[+]-1-[2-Pyrrolidinylmethyl]-pyrrolidine	C9H18N2	2070935	0.5629394
80	16.601	Diethylmalonic acid, di[5-methoxy-3-methylpentyl] ester	C21H40O6	1400314	0.3806454
81	16.667	2,5-Cyclohexadien-1-one, 4,4-dimethoxy-3-methyl-	C9H12O3	1153465	0.3135448
82	16.82	Methyl 4-methylcyclohexyl ethylphosphonate [isomer 1]	C10H21O3P	1224656	0.3328965
83	16.923	Cyclohexene, 2-butyl-1,3,3-trimethyl-	C13H24	1264508	0.3437295
84	16.967	1,3-Dioxolane-2,2-dipropanoic acid, dimethyl ester	C11H18O6	1443413	0.3923609
85	17.053	3-Methylbut-2-enoic acid, 4-methoxyphenyl ester	C12H14O3	784682	0.213299
86	17.093	1H-Indole, 2,3,5-trimethyl-	C11H13N	2941889	0.7996896
87	17.15	Silanol, dimethyl[1,1,2-trimethylpropyl]-	C8H20OSi	3452733	0.9385516
88	17.196	7-Methylhexahydrocyclopenta[c]pyran-1[3H]-one	C9H14O2	3277451	0.8909049
89	17.244	Nonane, 4,5-dimethyl-	C11H24	1146361	0.3116137
90	17.345	1H-Pyrrolizine, 2,3-dihydro-1-methoxy-7-[methoxymethyl]-	C10H15NO2	2872262	0.780763
91	17.377	Undecanoic acid	C11H22O2	2171455	0.5902636
92	17.408	3-Methylbut-2-enoic acid, 4-nitrophenyl ester	C11H11NO4	6229790	1.6934352
93	17.504	Heptylcyclohexane	C13H26	568314	0.154484
94	17.712	2-Ethoxy-4-methylaniline	C9H13NO	2753599	0.1416794
95	17.735	7,8-Epoxy-.alpha.-ionone	C13H20O2	1062884	0.2889223
96	17.773	Propanoic acid, 2-methylpropyl ester	C7H14O2	2268844	0.6167367
97	17.85	Pyridine, 3-methoxy-	C6H7NO	848558	0.2306623
98	17.901	Dihexyverine	C20H35NO2	690539	0.1877083
99	17.929	Phosphoric acid, 2-bromo-1-methylethenyl dimethyl ester	C5H10BrO4P	488421	0.1327668
100	18.133	2-Cyclohexyl-3-isopropyl-pent-4-en-2-ol	C14H26O	1409690	0.3831941
101	18.187	Ethanone, 1-[3-hydroxy-4-methoxyphenyl]-	C9H10O3	477399	0.1297707
102	18.241	Benzamide, N-tetrahydrofurfuryl-3-fluoro-	C12H14FNO2	453620	0.1233069
103	18.284	3-Methoxy-2[1H]-pyridone	C6H7NO2	519135	0.1411157
104	18.568	Dicyclohexyl propylphosphonate	C15H29O3P	776723	0.2111355
105	18.604	Dodecanoic acid	C12H24O2	4466726	1.2141839
106	18.674	Limonen-6-ol, pivalate	C15H24O2	1359760	0.3696217
107	18.811	Fumaric acid, ethyl 2-methylallyl ester	C10H14O4	630074	0.1712721
108	18.893	Cyclohexanecarboxylic acid, 4-methoxyphenyl ester	C14H18O3	439712	0.1195263
109	18.957	4-[2,7,7-Trimethylbicyclo[3.2.0]hept-2-en-1-yl]butan-2-one	C14H22O	509514	0.1385005
110	18.988	1-[benzo[d][1,3]dioxol-4-yl]-2-[dimethylamino]butan-1-one	C13H17NO3	675177	0.1835324
111	19.026	Succinic acid, monochloride, 4-methylpent-2-yl ester	C10H17ClO3	575846	0.1565314
112	19.072	Diethyl Phthalate	C12H14O4	734508	0.1996603
113	19.196	Fumaric acid, 2-ethylbutyl but-3-yn-2-yl ester	C14H20O4	984404	0.2675892
114	19.311	7-Ethyl-4-dodecen-6-one	C14H26O	602482	0.1637718
115	19.603	2-Hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone	C9H12O2	820868	0.2231354
116	19.706	2H-Pyran, tetrahydro-2-[[3-methyltricyclo[2.2.1.0[2,6]]hept-3-yl]oxy]-	C13H20O2	816513	0.2219516
117	19.721	Tridecanoic acid	C13H26O2	715917	0.1946067
118	19.741	Betazole	C5H9N3	1348581	0.3665829
119	19.899	[S]-[+]-6-Methyl-1-octanol	C9H20O	1215209	0.3303286
120	19.933	2-Propenamide, 3-ethoxy-N-phenyl-	C11H13NO2	2392799	0.6504312
121	20.049	Acetamide, N-[3-imidazol-1-ylpropyl]-2-	C9H15N3O2	2011722	0.5468436

	RT	Name	Formula	Area	Area Pct
122	20.181	methoxy- 5-Trimethylsilylpent-2-en-4-yne	C8H14Si	1435996	0.3903448
123	20.272	Ethane, 1-[3,4-dihydro-1H-benzo[c]pyran-1-yl] methylamino-2-[1-pyrrolidinyl]-	C16H24N2O	651519	0.1771015
124	20.361	Dodecyl isobutyl ether	C16H34O	452233	0.1229299
125	20.458	1,3-Cyclopentanedione, 2,4-dimethyl-	C7H10O2	1152172	0.3131933
126	20.499	Sulfurous acid, 2-ethylhexyl tridecyl ester	C21H44O3S	980091	0.2664168
127	20.558	[R]-3-Methyl-5-propylcyclohex-2-enone	C10H16O	3157079	0.8581844
128	20.621	Parapropamol	C9H11NO2	2880786	0.7830801
129	20.806	Phosphorus P4	P4	697297	0.1895453
130	20.834	Tetradecanoic acid	C14H28O2	5935091	1.6133275
131	20.859	Ethanone, 1-[2,3-dihydro-1,4-benzodioxin-6-yl]-	C10H10O3	742749	0.2019004
132	20.974	Cyclohexanamine, N,N-dimethyl-	C8H17N	714189	0.194137
133	21	L-Menthyl lactate	C13H24O3	477486	0.1297944
134	21.165	Loliolide	C11H16O3	3884114	0.10558133
135	21.442	3-Isopropyl-6,10-dimethylundecane-2-ol	C16H34O	572507	0.1556238
136	21.854	Pentadecanoic acid	C15H30O2	1083653	0.2945679
137	21.962	Caffeine	C8H10N4O2	1168630	0.3176671
138	21.973	Hexanedioic acid, mono[2-ethylhexyl]ester	C14H26O4	1485999	4.03937
139	22.056	1-Hexadecanol	C16H34O	809146	0.219949
140	22.166	1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl-	C20H38O	1250210	0.3398428
141	22.418	Phytol	C20H40O	1557884	0.4234774
142	22.488	13-Octadecenal, [Z]-	C18H34O	3272302	0.8895053
143	22.499	Hexadecanoic acid, methyl ester	C17H34O2	2328108	0.6328464
144	22.898	n-Hexadecanoic acid	C16H32O2	30474569	8.2838597
145	22.971	Dibutyl phthalate	C16H22O4	3940618	1.0711727
146	23.641	4[1H]-Pyrimidinone, 2,6-dimethyl-	C6H8N2O	43747911	11.8919338
147	23.67	Pyridine, 2-ethyl-6-methyl-	C8H11N	619192	0.1683141
148	24.015	Oct-3-enoic acid, undec-2-en-1-yl ester	C19H34O2	1447751	0.3935401
149	24.183	13-Octadecenoic acid, methyl ester	C19H36O2	1955391	0.5315312
150	24.396	Methyl stearate	C19H38O2	553206	0.1503772
151	24.412	4[1H]-Pyrimidinone, 2,6-dimethyl-	C6H8N2O	3958947	1.0761551
152	24.535	Oleic Acid	C18H34O2	3282665	0.8923223
153	24.588	2[a]-Methoxymethyl-1,2,5-trimethylpiperidin-4[a]-ol	C10H21NO2	567349	0.1542217
154	24.72	Octadecanoic acid	C18H36O2	1166037	0.3169622
155	25.275	3'-Acetyllycopsamine	C17H27NO6	3668628	0.997238
156	30.132	bis-[4,6-Di-azido-1,3,5-triazin-2-yl]-amine	C6HN19	683699	0.1858489
157	31.348	Silane, dimethyl[4-methoxyphenoxy]tridecyloxy-	C22H40O3Si	692427	0.1882215
158	31.59	Stigmasta-4,7,22-trien-3.beta.-ol	C29H46O	847528	0.2303824
159	32.075	Stigmastan-6,22-dien, 3,5-dedihydro-	C29H46	1554678	0.422606
160	32.92	5-Docosyldihydrofuran-2[3H]-one	C26H50O2	25625511	6.965747
161	33.556	Campesterol	C28H48O	512910	0.1394236
162	33.896	Stigmasterol	C29H48O	906993	0.2465466
163	33.999	Stigmasta-4,22-dien-3-ol, 4-methyl-, [3.beta.]-	C30H50O	522274	0.141969
164	34.581	.gamma.-Sitosterol	C29H50O	2233837	0.6072208
165	34.696	Stigmasta-3,5-dien-7-one	C29H46O	1620475	0.404915
166	35.323	Trifluoroacetic acid, pentadecyl ester	C17H31F3O2	2771426	0.7533529
167	35.802	Aciphyllene	C15H24	566245	0.1539216

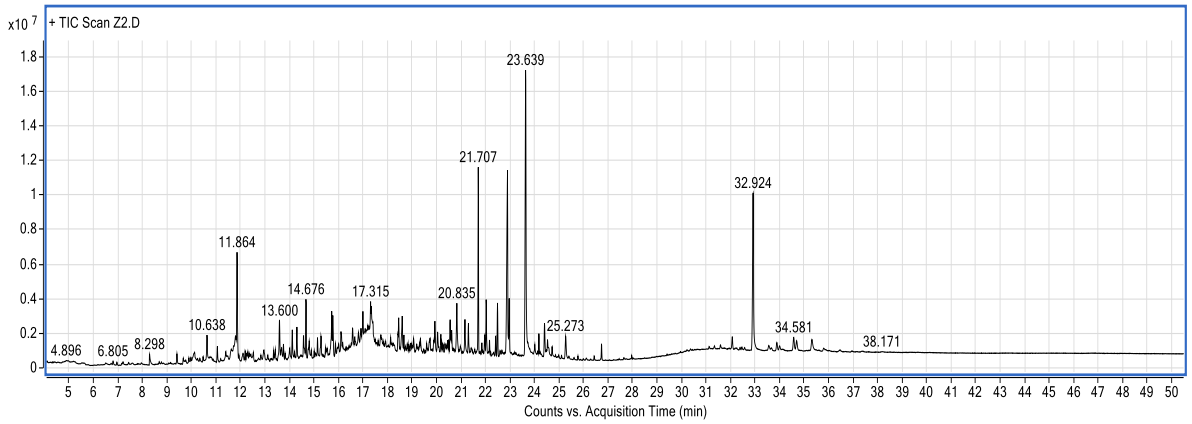

Figure 4. Mass spectrum of separated compounds of *H. curassavicum* by GC-MS.

3.6. Chemical Compounds Shared by the Studied Species

From observing the results, we found that the four studied species share some chemical compounds, with each other, but each species has many of its own compounds, as shown in Table 6. Considerable variations in the types and amounts of these phytochemicals might demonstrate their similarity or difference.

Table 6. Chemical compounds shared by the studied species.

	Chemical compounds	*H. europaeum*	*H. crispum*	*H. bacciferum*	*H. curassavicum*
.1	.gamma.-Sitosterol	+	+	+	+
.2	1,2-Benzenedicarboxylic acid, bis[2-methylpropyl] ester	+	+	+	–
.3	13-Octadecenoic acid, methyl ester	+	+	+	+
.4	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	+	–	–	+
.5	1H-Pyrrolizine, 2,3-dihydro-1-methoxy-7-[methoxymethyl]-	–	+	+	+
.6	1H-Pyrrolizine-7-methanol, 2,3,5,7a-tetrahydro-1-hydroxy-, [1S-cis]-	–	+	+	–
.7	1-Methyl-5-fluorouracil	+	+	+	+
.8	2,3-Butanediol, 2,3-dimethyl-	–	+	+	–
.9	2,4-Dihydroxy-2,5-dimethyl-3[2H]-furan-3-one	+	–	–	+
.0	2[4H]-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, [R]-	+	+	+	–
.1	2-[Hydroxymethyl]-6-methylpyridin-3-ol	–	+	+	–
.2	2-Ethoxy-4-methylaniline	–	+	+	+
.3	2-Hydroxy-2,6-dimethyl-hept-6-en-3-one	+	+	+	–
.4	2-Hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone	+	–	–	+
.5	2-Pentadecanone, 6,10,14-trimethyl-	+	+	+	–
.6	3'-Acetyllycopsamine	+	+	+	+
.7	3-Ethyl-2,6,10-trimethylundecane	–	+	+	–
.8	3-Hexanol, 2-methyl-	+	–	–	+
.9	3-Methylbut-2-enoic acid, 4-nitrophenyl ester	+	–	–	+
0	3-Pyridinamine, 2,6-dimethyl-	–	+	+	–
1	4,6-Diamino-O-cresol	–	+	+	–
2	4-Methyl-2,4-bis[p-hydroxyphenyl]pent-1-ene, 2TMS derivative	–	+	+	–
3	6-Azathymine, Bis[methyl] ether	–	+	+	–
4	9,12,15-Octadecatrienoic acid, [Z,Z,Z]-	–	+	+	–
5	9-Octadecenoic acid, [E]-	–	+	+	–
6	Aziridine, 2-phenyl-	+	+	+	+
7	Benzamide, 4-fluoro-N-allyl-	+	+	+	–
8	Benzeneacetic acid	+	–	–	+

	Chemical compounds	*H. europaeum*	*H. crispum*	*H. bacciferum*	*H. curassavicum*
29	Benzeneethanol, 2-amino-6-methoxy-	–	+	+	–
30	Benzoic acid	+	+	+	–
31	Benzoic acid, 4-amino-, methyl ester	–	+	+	–
32	Bicyclo[3.2.1]octane	–	+	+	–
33	Borazine, 2,4,6-trimethyl-	+	+	+	–
34	Butanoic acid, 2-hydroxy-2-[1-methoxyethyl]-3-methyl-, [2,3,5,7a-tetrahydro-1-hydroxy-1H-pyrrolizin-7-yl]methyl ester, [1S-[1.alpha.,7[2R*,3S*],7a.alpha.]]-	+	+	+	–
35	Butanoic acid, 2-oxo-, methyl ester	–	+	+	–
36	cis-7,cis-11-Hexadecadien-1-yl acetate	–	+	+	–
37	Dibutyl phthalate	+	+	+	+
38	D-Limonene	–	+	+	–
39	Dodecanoic acid	+	+	+	+
40	Hexadecanoic acid, methyl ester	+	+	+	+
41	Hexanedioic acid, bis[2-ethylhexyl] ester	+	+	+	–
42	Hexanedioic acid, mono[2-ethylhexyl]ester	+	–	–	+
43	Hydroxymethyl 2-hydroxy-2-methylpropionate	+	+	+	–
44	Indole	+	+	+	+
45	Loliolide	+	+	+	+
46	L-Valinamide, N-[methoxycarbonyl]-	–	+	+	–
47	N,N'-Bis[2,6-dimethyl-6-nitrosohept-2-en-4-one]	–	+	+	–
48	n-Decanoic acid	+	+	+	–
49	n-Hexadecanoic acid	+	+	+	+
50	Octadecanoic acid	+	+	+	+
51	Pentadecanoic acid	+	–	–	+
52	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	–	+	+	–
53	Phthalic acid, butyl 3-phenylpropyl ester	–	+	+	+
54	Phytol	+	+	+	+
55	Propane, 2-fluoro-2-methyl-	+	+	+	–
56	Silane, dichloromethylpentyl-	–	+	+	–
57	Tetradecanoic acid	+	+	+	+
58	Tricyclo[4.3.1.0[2,5]]decane	–	+	+	–

+: Present - : Missing Red color: Common among the studied species.

4. Discussion

The results of this study showed that *H. curassavicum* contains 167 species compared to other species because it grows in areas with silty clay soils that have the ability to retain water, salts and nutrients compared to *H. bacciferium* and *H. crispum*, which contain 61 and 60 compounds respectively, which grow in desert areas with sandy soils that have high porosity, allowing water and organic materials to penetrate to the depths compared to clay and silty soils that have low permeability, and are characterized by their ability to retain water and essential nutrients for plants [23]. The species *H. europaeum*, contains 102 compounds, which is greater than the amount of the two aforementioned compounds because it grows in mountainous areas with calcareous soils rich in organic and mineral materials, and because soils are the basic environment on which plants depend on their formation, survival and continuity because they contain water and nutrients [24].

The current study clarified the relationship between these four species, whether they were distant or closely related from each other. The results of the study showed that all species shared 14 compounds, which indicates the strength of the connection between the species. It was also found that *H. bacciferum* and *H. crispum,* have the same active compounds, which is 2,3-Butanediol, 2,3-dimethyl-, in addition to the presence of 20 chemical compounds found in both species and not found in the other two species, which confirms closely related between them. This confirms that Al-Rudainy [18]

concluded, that they fell into one secondary group, as shown in Figure [5]. This also reflects the great similarity between the two species, at a time when the isolation of each of them with its own compounds confirms the strength of its isolation as an independent species. We also found that *H. europaeum* shares 12 compounds with the two aforementioned species, and this may be due to its genetic proximity to them compared to the species *H. curassavicum*, despite the differences in the environmental conditions in which they exist, while *H. bacciferum, H. crispum*, and *H. curassavicum* growing in Basrah Governorate have only three compounds that are not found in the fourth species growing in northern Iraq. This indicating that closely related species have more similar chemical compounds than more distantly related species which is consistent with what Rønsted [5] found. This may indicate that genetic relationship is stronger than the surrounding environmental conditions.

The results showed the presence of a number of pyrrolizidine alkaloids in the species under study, which are among the compounds famous in the genera of the Boraginaceae family [25]. These are compound 3'-Acetyllycopsamine in the four species, compound 1H-Pyrrolizine, 2,3-dihydro-1-methoxy-7-[methoxymethyl]- in *H. bacciferum, H. crispum* and *H. curassavicum*, the compound 1H-Pyrrolizine-7-methanol, 2,3,5,7a-tetrahydro-1-hydroxy-, [1S-cis]- in *H. bacciferum* and *H. crispum* and Echimidine in *H. europaeum.*

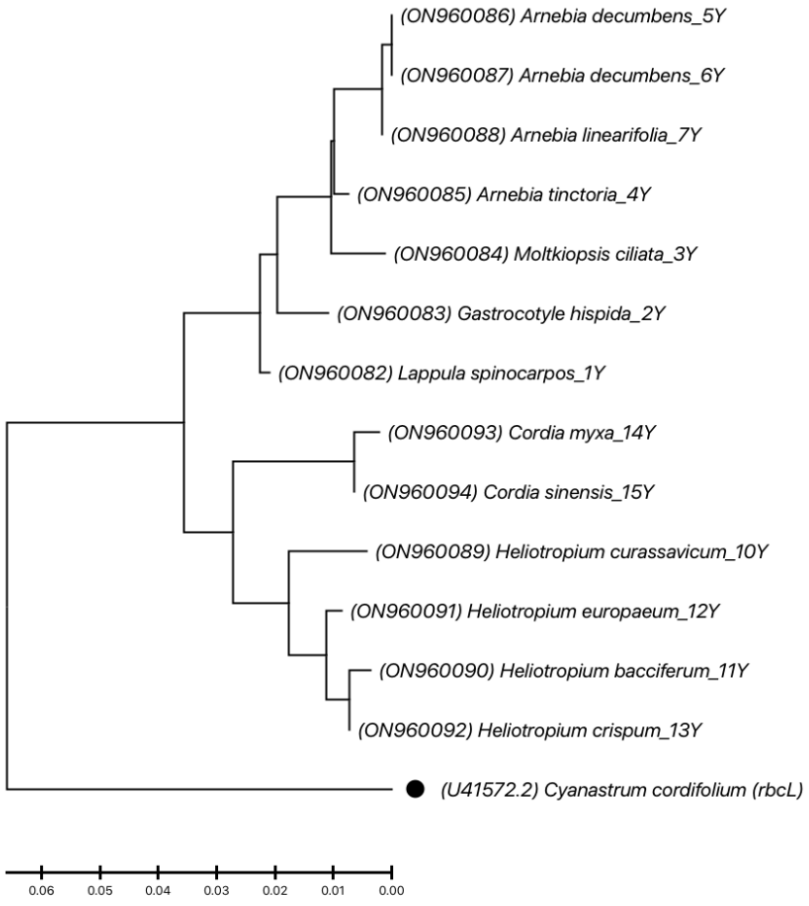

Figure 5. A cluster analysis diagram to illustrate the genetic distance between the studied species. Al-Rudainy [18].

Conclusion

Each of the four species of the *Heliotropium* possesses many unique chemical compounds not found in the other species, which constitutes an important taxonomic characteristic .The environment has a significant influence on the chemical content of the studied species, both quantitatively and qualitatively. Phenotypically and genetically similar species possess many common chemical compounds.

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